SUPPLEMENTAL INFORMATION
for

*In Vitro* Biotransformation of Dimethylarsinic Acid and Trimethylarsine Oxide by Anaerobic Microflora of Mouse Cecum Analyzed by HPLC-ICP-MS and HPLC-ESI-MS

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Four supplemental figures are included in this document with their respective captions:

**Figure SI-1**
**Figure SI-2**
**Figure SI-3**
**Figure SI-4**
**Figure SI-1:** Example HPLC-ICP-MS chromatograms (m/z 75) for various mixtures of arsenic standard. A) HPLC-ICP-MS using **Separation 1** for the separation of TMAS standard (upper trace) with TMAO as an impurity and DMDTA standard (lower trace) with DMTAV and DMAV as impurities. * denotes elution time of As\textsuperscript{III}, As\textsuperscript{V}, and MMA\textsuperscript{V} elute. B) HPLC-ICP-MS using **Separation 2** for the separation of As\textsuperscript{III}, DMA\textsuperscript{V}, MMA\textsuperscript{V}, DMTAV, As\textsuperscript{V}. C) HPLC-ICP-MS using **Separation 3** for the separation of a standard mixture of DMDTA, DMTAV, and TMAS. ** denotes elution time of As\textsuperscript{III}, MMA\textsuperscript{V}, DMA\textsuperscript{V}, and As\textsuperscript{V}. The conditions for each separation are listed in **Table 1**.
Intestinal ceca of B6C3F1 male mice were removed under sterile anaerobic conditions. Mixed with VPI buffer (0.1g CaCl$_2$, 0.2g MgSO$_4$, 0.5g KH$_2$PO$_4$, 5.0g NaHCO$_3$ and 1.0g NaCl l$^{-1}$) at 0.03g cecal contents per mL of buffer. Fortified with DMA and incubated in anaerobic chamber according to the Supplementation Table. Flash frozen using liquid N$_2$, stored at -72 °C until analysis.

Thawed and vortexed, then centrifuged at 10400 x g for 10 min. Diluted appropriately with 20mM (NH$_4$)$_2$CO$_3$ at pH – 9.0 to minimize conversion of oxide to sulfide while awaiting analysis.

**Supplementation Table**

<table>
<thead>
<tr>
<th>Supplementation level (ng As g$^{-1}$)</th>
<th>Incubation Time (Hours at 37 °C)</th>
<th>DMA$^V$ Supplementation</th>
<th>TMAO Supplementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>I 0</td>
<td>0</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>II 20</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>III 200</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>IV 1000</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Controls (no cecum, VPI buffer) at t = 0 and 24 only. All controls supplemented at all four levels (n = 3).

**Figure SI-2:** Summary of the experimental design including: cecal content preparation, supplementation levels (DMA$^V$ and TMAO), anaerobic incubation period, and sample preparation prior to analysis.

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Figure S1-3: Time dependent metabolism of DMA$^\text{V}$ (A) 20 ng As g$^{-1}$, B) 1000 ng As g$^{-1}$ (B1, major metabolites, B2, minor metabolites) in incubated reaction mixtures containing the anaerobic microflora from a mouse cecum. Data obtained by HPLC-ICP-MS analysis using Separation 1. Error bars represent 1σ in the positive direction. Time dependence for concentrations of sum of all arsenic species (---), DMA$^\text{V}$ (--), DMTA$^\text{V}$ (--), and DMDTA (--), iAs (--$>$95% As$^\text{V}$) and TMAS (--).
Figure SI-4: Time dependent metabolism of TMAO (A) 17 ng As g⁻¹, B) 830 ng As g⁻¹) in incubated reaction mixtures containing the anaerobic microflora from a mouse cecum. Data obtained by HPLC-ICP-MS analysis using Separation 1. Error bars represent 1σ in the positive direction.
Time dependence for concentrations of sum of all arsenic species (---■---), TMAS (---●---), TMAO (---○---), and iAs (---▲---) (>95% AsV).